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Role of Sucrose Phosphate Synthase in Sucrose Biosynthesis in Ripening Bananas and Its Relationship to the Respiratory Climacteric[1]

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ABSTRACT

During ripening of bananas (*Musa* spp. [AAA group, Cavendish subgroup]), there is a massive conversion of starch to sucrose. Also during ripening there is a rise in respiration known as the respiratory climacteric. In this study changes in carbohydrate content, activities of starch and sucrose metabolizing enzymes, and respiration were measured to assess their potential interrelationships. Sucrose phosphate synthase activity increased dramatically during the first 4 days after initiation of ripening by ethylene treatment. Starch concentration decreased and sucrose concentration increased during this time period. Developmental changes in sucrose phosphate synthase activity were measured with limiting substrate (plus Pi) and saturating substrate concentrations. Activities were not parallel under the two assay conditions, providing tentative evidence that kinetically different forms of the enzyme may exist at different stages of ripening. Sucrose accumulation rate was most highly correlated with sucrose phosphate synthase activity assayed with limiting substrate concentrations (plus Pi). The cumulative amount of CO_2 respired during ripening was positively correlated with sugar accumulation (R^2 = 0.97). From this linear regression it was calculated that a constant 0.605 millimoles of CO_2 was evolved per mole of sucrose formed throughout ripening. Using this quantity, the percentage of the total respiratory ATP produced which was required for the conversion of starch to sucrose was calculated assuming different models for carbon export from the amyloplast. The results suggest that sucrose biosynthesis during ripening constitutes a significant sink for respiratory ATP.

Bananas accumulate soluble sugars in postharvest conditions from a stored starch reserve, unlike many fruits which are dependent upon concurrent photosynthesis for sugar accumulation. Starch, which constitutes 20 to 25% of the fresh weight of unripe bananas, is almost entirely converted to soluble sugars during ripening with approximately 2 to 5% lost as CO_2 in respiration (6, 25, 27). Initially the predominant sugar is sucrose. Hexose sugars appear after sucrose, and ultimately exceed sucrose concentration.

During ripening, bananas undergo a respiratory climacteric. Mitochondria of climacteric fruit remain tightly coupled throughout ripening (4, 31, 37) and presumably provide the necessary ATP for the synthesis of UDP-Glc, a substrate required for sucrose synthesis.

The rapid increase in respiration and conversion of starch to sugars during banana ripening are indicative of simultaneous increases in both glycolytic and gluconeogenic carbon flow. Based upon the rate of conversion of total carbohydrates to CO_2 and soluble sugars during banana ripening, Beaudry *et al.* (5) estimated that flux through the glycolytic pathway increased 4- to 5-fold and gluconeogenic flux increased 50- to 100-fold. Despite the estimated 50- to 100-fold increase in gluconeogenesis, Glc 6-P[2] and Fru 6-P (gluconeogenic intermediates) increased only 2-fold. It was therefore suggested (5) that there was an increase in some enzymatic step(s) between starch degradation and the appearance of hexose-phosphates in the amyloplast and/or between hexose-phosphates and sucrose synthesis in the cytosol. However, Beaudry *et al.* (5) did not identify any increase(s) in enzyme activity in bananas of the magnitude required to support their conclusion.

Starch degradation in bananas appears to be mediated primarily by phosphorylase (1, 29, 36). Early studies with bananas (29, 36) reported a substantial increase in phosphorylase activity associated with the starch to sugar conversion. Enzyme extraction procedures in bananas have since improved (2), and it now appears that phosphorylase activity of unripe bananas is sufficient to account for the rapid loss of starch content during ripening (1). With regard to sucrose synthesis, Terra *et al.* (33) reported an increase in sucrose synthase activity during ripening and suggested that sucrose synthase may play a major role in the regulation of sucrose accumulation. SPS[2], the more probable enzyme involved in sucrose synthesis, has been reported in bananas, but the activity was low (5.7 μmol h^{-1} [g fresh weight]$^{-1}$) and the stage of ripeness was not indicated (2, 28). SPS has recently been shown to be responsible for sucrose accumulation in ripening muskmelon fruit (13) and in leaf tissues of some species the enzyme is allosterically regulated (8). Therefore, if there is an activated step between hexose-phosphates and sucrose as suggested by Beaudry *et al.* (5), SPS would be a likely candidate.

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[2] Abbreviations: SPS, sucrose phosphate synthase; Glc-6-P, glucose 6-P; Fru-6-P, fructose 6-P.

In the present study, changes in carbohydrate concentration, enzyme activities associated with starch and sucrose metabolism, and respiration were measured during banana ripening. The ATP requirement necessary for uridylate turnover during sucrose biosynthesis and its relationship to the respiratory climacteric are discussed.

MATERIALS AND METHODS

Plant Material

Mature, green banana (*Musa* spp. [AAA group, Cavendish subgroup]) fruit were obtained from a grocery store warehouse prior to treatment with ethylene gas. Hands were placed in a sealed chamber with an atmosphere containing 1000 ppm ethylene in air. The chamber was aerated, resealed, and 1000 ppm ethylene reestablished every 12 h when treatment lasted 36 h. In studies involving 18 h ethylene treatment, the chamber was aerated, resealed, and 1000 ppm ethylene reestablished after 9 h.

In a 12 d study of fruit carbohydrates and enzyme activities, fruit were exposed to ethylene for 36 h and ripened at 25°C. Fruit were sampled immediately prior to exposure to ethylene and every 48 h thereafter. There were four hands from which fruit were sampled, each constituting a replication. A segment, approximately 2 cm long, was cut from the midsection of the fruit. Individual segments were peeled, sliced, and frozen immediately in liquid N_2. Samples were powdered in liquid N_2 using a mortar and pestle, and stored at −80°C.

Fruit respiration and carbohydrates were measured in two separate experiments. In those studies, bananas were exposed to ethylene for 18 h and ripened at 25°C. Samples were taken three to four times daily over a 5 d ripening period. Segments, 1.5 cm long, were cut from the midsection of the fruit, peeled, and weighed. After respiration was measured, the segments were sliced, pooled, and frozen in liquid N_2. Samples were powdered and stored, as described above.

Carbohydrate Analysis

All samples were extracted three times with hot 80% (v/v) ethanol. An aliquot of the supernatant was dried *in vacuo* at 40°C and resolubilized in water. Sucrose and hexose sugar concentrations were determined enzymatically by the method of Jones *et al.* (17). The insoluble residue remaining after ethanolic extraction was resuspended in 2 mL of 30 mM HCl and boiled for 30 min. After cooling, the pH was adjusted to pH 4.5 using KOH. The gelatinized starch was digested for 60 min at 55°C using approximately 36 units amyloglucosidase from *Aspergillus oryzae*. The amyloglucosidase had previously been dialyzed against 50 mM Na-acetate buffer (pH 4.5). Samples were boiled for 2 min to stop the reaction. After cooling and centrifugation, an aliquot of the supernatant was used to measure glucose in a solution (1 mL) containing 100 mM Hepes-NaOH (pH 8.0), 5 mM $MgCl_2$, 1 mM NAD, 1 mM ATP, 5 mM DTT, 2.5 units hexokinase (from Bakers yeast), and 2.5 units Glc 6-P dehydrogenase (from *Leuconostoc mesenteroides*). The reaction mixture was incubated at 25°C for 30 min and absorbance at 340 nm was measured.

Enzyme Extraction

Frozen banana fruit tissues were ground using a 1:10 tissue-to-buffer ratio in a chilled mortar and pestle. The extraction buffer contained 100 mM Mops-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mg mL^{-1} BSA, 2% (v/v) glycerol, 0.05% (v/v) Triton X-100, 2.5 mM DTT, and 2% (w/v) PVPP. Homogenates were centrifuged at 30,000*g* for 10 min, except in the case of SPS assays, in which homogenates were centrifuged for 1 min at 10,000*g*. Supernatants were desalted by centrifugal filtration (12) on Sephadex G-25-50 columns equilibrated with 100 mM Mops-NaOH (pH 7.5), 5 mM $MgCl_2$, 2.5 mM DTT, and 0.5 mg mL^{-1} BSA.

Ball and ap Rees (3) reported poor recoveries of the regulatory metabolite fructose 2,6-bisphosphate in unripe bananas. To determine if recovery was a problem in extraction of enzyme activities measured in this study, samples of unripe and ripe banana tissues were mixed prior to extraction. Samples were ground, desalted, and assayed for SPS, sucrose synthase, and acid and neutral invertase. Measured activity of the mixed extracts was the mean of the activity in the extracts measured separately for each enzyme.

Starch and Maltose Phosphorylase Assay

Reaction mixtures (1 mL) contained 50 mM Hepes-NaOH (pH 7.0), 10 mM Na-phosphate, 0.1 mg mL^{-1} BSA, 0.4 mM NAD, 2 units phosphoglucomutase, 5 units Glc 6-P dehydrogenase (from *L. mesenteroides*), and 50 μL desalted extract. To measure starch phosphorylase activity, 1 mg soluble starch was included in the reaction mixture. Maltose phosphorylase activity was measured using 20 mM maltose. Reactions were run at 25°C and monitored spectrophotometrically at 340 nm.

SPS Assay

SPS activity was measured under limiting (plus Pi) and saturating substrate concentrations. Under limiting substrate conditions, 45 μL of desalted extract was incubated with a 25 μL mixture containing 50 mM Mops-NaOH (pH 7.5), 15 mM $MgCl_2$, 3 mM Fru 6-P, 9 mM Glc 6-P, 10 mM UDP-Glc, and 10 mM Pi. Reaction mixtures of saturating substrate concentrations consisted of 50 mM Mops-NaOH (pH 7.5), 15 mM $MgCl_2$, 5 mM Fru 6-P, 15 mM Glc 6-P, and 10 mM UDP-Glc. Reaction mixtures were incubated at 25°C and terminated at 0 and 15 min with 70 μL of 30% KOH. All remaining Fru 6-P and Fru was destroyed by placing tubes in boiling water for 10 min. After cooling, a 1 mL mixture of 0.14% anthrone in 13.8 M H_2SO_4 was added. Following a 20 min incubation at 40°C tubes were cooled and color development was measured at 620 nm.

Acid Invertase Assay

Reaction mixtures (100 μL) to determine acid invertase activity contained desalted extract (40 μL), 100 mM citrate-phosphate (pH 5.0) and 120 mM sucrose. Reactions were incubated at 25°C and terminated by boiling for 30 s at 0 and 30 min. Hexose sugar concentration was determined enzymatically as described previously (17).

Sucrose Synthase and Neutral Invertase Assay

To measure neutral invertase activity, reaction mixtures (1 mL) consisted of 50 mM Hepes-NaOH (pH 7.0), 2 mM $MgCl_2$, 1 mM EDTA, 15 mM KCl, 5 units Glc 6-P dehydrogenase (from *L. mesenteroides*), 4 units hexokinase (from Bakers yeast), 4 units phosphoglucoisomerase, 1 mM ATP, 0.4 mM NAD, 50 mM sucrose, and 50 μL desalted extract. The addition of 1 mM UDP initiated the sucrose synthase assay. Reactions were monitored spectrophotometrically at 340 nm.

Respiratory Measurements

Fruit respiration was measured in a closed system using a LiCor model 6000 Portable Photosynthesis Meter[3] (LiCor, Lincoln, NE 68504). A 4 L chamber from which the upper layer of plastic line was removed, was used. Banana slices were supported on the remaining plastic line within the chamber such that circulation of gasses was not impaired.

Initial tests were conducted using fruit purchased from a local market to test the validity of using sliced, peeled, banana segments in a LiCor Photosynthesis Meter to measure respiration. Measurements were made using whole fruit, with and without the peel, and fruit slices without the peel. CO_2 evolution from each was measured for 1 h at 5 min intervals. In all further studies of banana respiration, three segments (sampled as described above) were placed in the chamber and, after 30 min, CO_2 evolution was measured. The mean of measurements taken for 30 additional min at 5 min intervals was used to determine respiration rate. Respiration rates and carbohydrate concentrations from the two studies were similar and were, therefore, combined to form a single data set. The data treated in this manner provided 30 short sampling intervals during the five day experiment.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the North Carolina Agricultural Research Service or the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

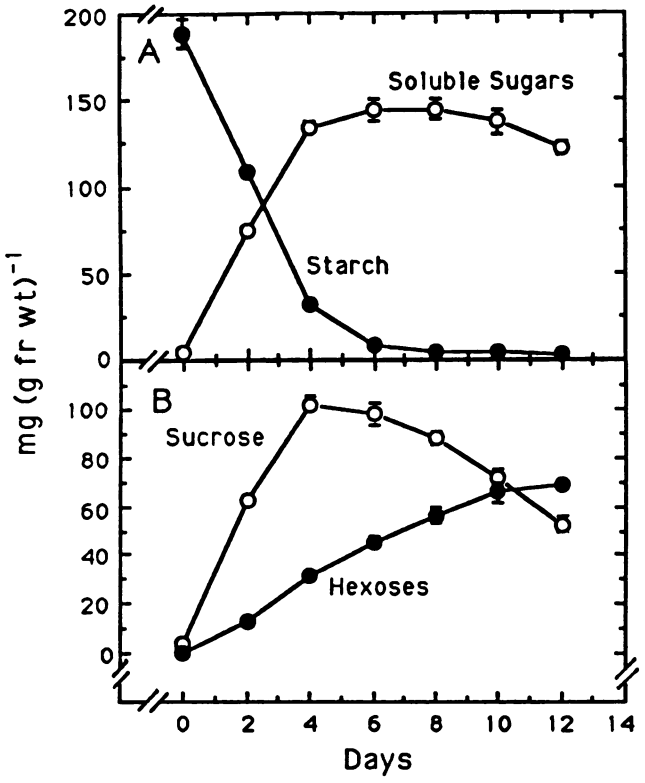

Figure 1. Concentrations of (A) starch (●) and soluble sugars (○); and (B) sucrose (○) and hexose sugars (●) in ripening banana fruit. Fruit were sampled prior to 36 h exposure to 1000 ppm ethylene, and every 2 d thereafter. Data points are the mean of four replications, ±1 SE.

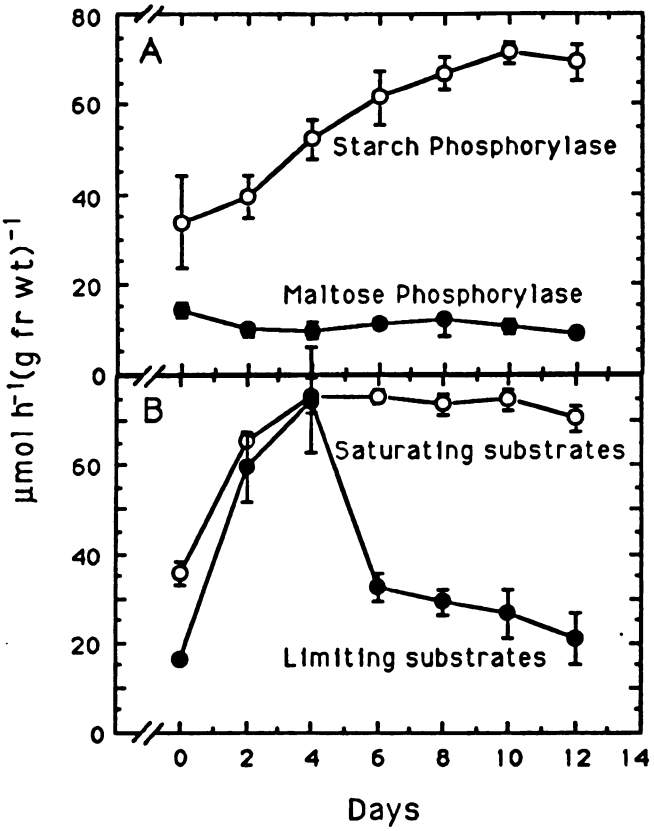

Figure 2. A, Starch phosphorylase (○) and maltose phosphorylase (●) activities; B, SPS activity assayed under saturating (○) and limiting (●) substrate concentrations (plus Pi) in ripening banana fruit. Fruit were sampled prior to 36 h exposure to 1000 ppm ethylene, and every 2 d thereafter. Data points are the mean of four replications, ± 1 SE.

Correlation between Respiration and Soluble Sugar Accumulation

To evaluate the potential relationship between respiration rate and sugar accumulation, the data were expressed as mmol kg^{-1} for each sampling interval. Because our data and reports of others (5, 23, 32, 35) indicate that hexose sugars arise from sucrose hydrolysis, the soluble sugar concentration at the midpoint of each sampling interval was calculated and expressed as sucrose equivalents for each sampling interval.

In order to express respiration rate as mmol CO_2 kg^{-1} graphical integration was used. Respiration rate, mmol CO_2 kg^{-1} h^{-1}, was plotted as a function of hours after initiation of ethylene treatment. From the resulting plot, the cumulative area under the curve during each sampling interval was measured using a LiCor LI-3100 area meter (LiCor). Using a known area from a similar plot as reference, the cumulative mmol CO_2 kg^{-1} evolved over time was calculated. The cumulative mmol CO_2 kg^{-1} evolved during consecutive sampling intervals was regressed against the concentration of total soluble sugars, mmol sucrose kg^{-1}, and the correlation was determined.

RESULTS

Changes in Carbohydrates and Enzyme Activities in Ripening Banana Fruit

The decline in starch concentration during the first 4 d after ethylene treatment (Fig. 1A) was accompanied by an increase in soluble sugar concentration (Fig. 1B) as has been reported previously (6, 23, 25, 27, 33). Starch phosphorylase activity was high prior to ethylene treatment (Fig. 2A). Although activity of starch phosphorylase increased during ripening, the increase would not have been necessary to account for the starch breakdown which occurred during the first 4 d.

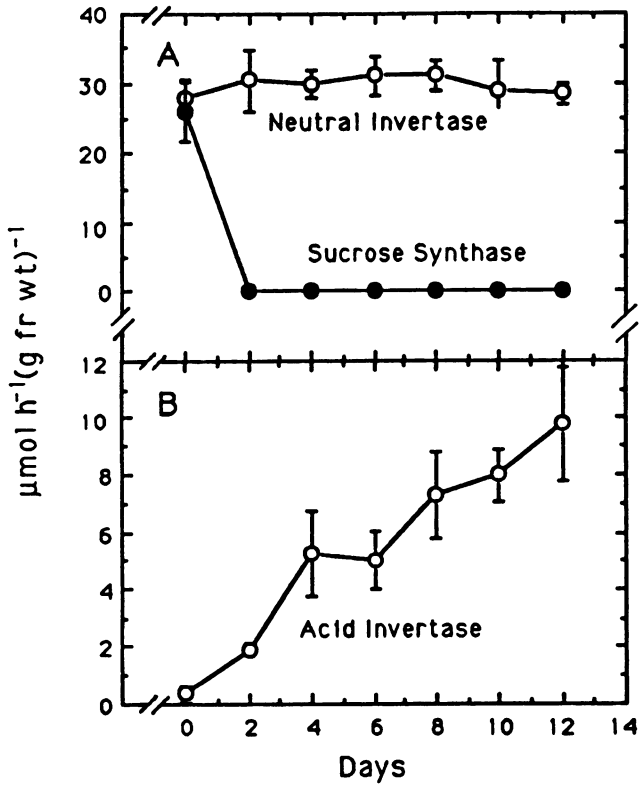

Figure 3. A, Neutral invertase (○) and sucrose synthase (●) activities; B, acid invertase activity in ripening banana fruit. Fruit were sampled prior to 36 h exposure to 1000 ppm ethylene, and every 2 d thereafter. Data points are the mean of four replications, ±1 SE.

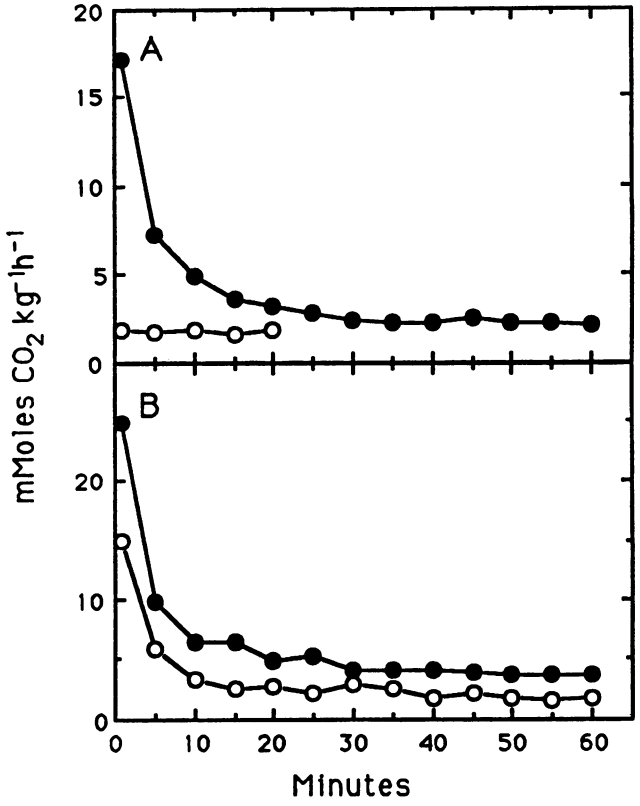

Figure 4. Respiration of (A) whole bananas with (○) and without (●) the peel; and (B) three peeled banana slices from unripe (○) and ripening (●) fruit, measured in a LiCor Photosynthesis Meter. Measurements were taken immediately after fruit were peeled and/or sliced for 60 min at 5 min intervals.

However, the increase in soluble sugars, which during the first 4 d was predominantly sucrose (Fig. 1B), appeared to result from the increase in SPS activity (Fig. 2B).

SPS activity was present prior to ethylene treatment and increased dramatically to 75 μmol h^{-1} (g fresh weight)$^{-1}$ during the first 4 d, whether measured under saturating or limiting substrate concentrations (Fig. 2B). Under saturating substrate concentrations, SPS activity remained at approximately 75 μmol h^{-1} (g fresh weight)$^{-1}$ from 4 d to the final sampling. However, SPS activity under limiting substrate concentrations (plus Pi) declined sharply from 75 μmol h^{-1} (g fresh weight)$^{-1}$ to about 30 μmol h^{-1} (g fresh weight)$^{-1}$ at 6 d, where it remained through the final sampling. The different developmental profiles of SPS activity measured under the two assay conditions may indicate a change in the activation state of the enzyme during banana ripening (32).

Activities of enzymes responsible for sucrose degradation changed during banana ripening. Sucrose synthase activity was relatively high (25 μmol h^{-1} [g fresh weight]$^{-1}$) prior to ethylene treatment, but was undetectable in fruit sampled thereafter (Fig. 3A). Neutral invertase activity remained constant at 30 μmol h^{-1} (g fresh weight)$^{-1}$ (Fig. 3A), while acid invertase activity increased steadily (Fig. 3B) throughout the

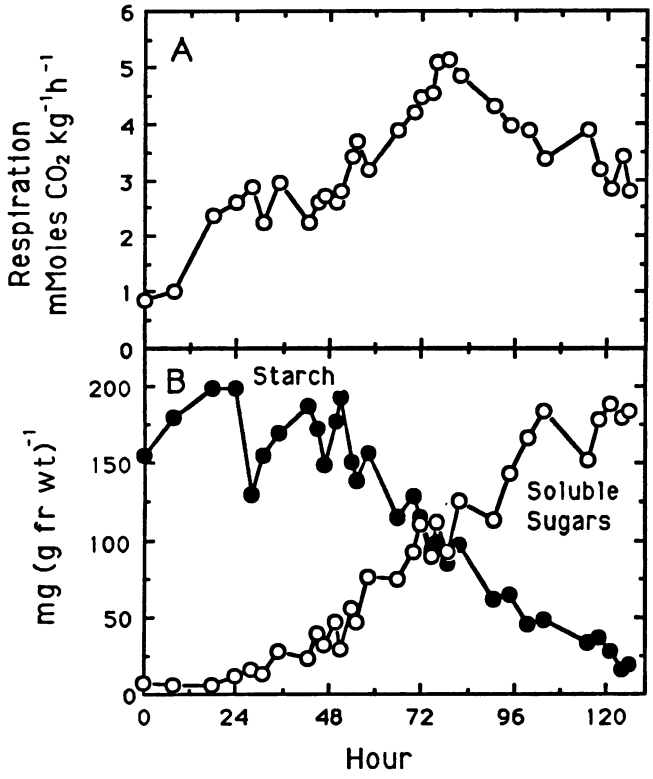

Figure 5. A, Respiration rate, and B, concentrations of starch (●) and soluble sugars (○) of banana fruit slices from three individual fruits. Fruit were exposed to 1000 ppm ethylene from 0 to 18 h and sampled through 125 h. Data from two studies were combined.

sampling period. The increase in acid invertase was associated with a decline in the sucrose pool and concomitant increase in hexose sugar concentration (Fig. 1B), which was comprised of roughly equimolar Glc and Fru (data not shown). These results support the postulate that the hexose sugars arise from sucrose hydrolysis (5, 23, 32, 36).

Fruit Respiration

Preliminary studies indicated that peeled banana slices could be used to measure fruit respiration in a LiCor Photosynthesis Meter. Respiration rates measured in this way were similar to reported values for bananas (11, 25, 36). Respiration rate of intact fruit was approximately 2 mmol CO_2 kg^{-1} h^{-1} (Fig. 4A). Respiration rate of the same fruit increased dramatically when the peel was removed but rapidly declined. After 30 min, respiration of the peeled fruit had reached a steady state which was comparable to the rate measured prior to peeling. Respiration of peeled banana slices was also high initially but soon declined, and within 30 min a steady state rate was achieved (Fig. 4B). There was some variability in measured steady state values; therefore, in all subsequent studies CO_2 evolution was measured in slices at 5 min intervals between 30 and 60 min after sampling and mean values were calculated.

Measured respiration rates in bananas increased from less than 1 mmol CO_2 kg^{-1} h^{-1} to a steady rate of 2.5 mmol CO_2 kg^{-1} h^{-1} after exposure to ethylene (Fig. 5A). At approximately 48 h, the climacteric rise was initiated. The climacteric peak of 5 mmol CO_2 kg^{-1} h^{-1} occurred at 75 h, after which time respiration declined. The onset of the climacteric rise at 48 h, coincided with the initiation of starch degradation and accumulation of soluble sugars (Fig. 5B).

The cumulative amount of CO_2 evolved (mmol CO_2 kg^{-1}) for each sampling interval was correlated (R^2 = 0.97) with the accumulation of soluble sugars (Fig. 6). The data include each interval from the two combined respiration studies over the 125 h ripening period.

DISCUSSION

Changes in SPS Activity and Sucrose Accumulation

The conversion of starch to sucrose during the first 4 d after ethylene treatment was associated with an increase in SPS activity (Figs. 1, A and B, and 2B). The increase in both SPS activity and sucrose concentration, as well as the lack of detectable sucrose synthase activity indicate that SPS mediates sucrose biosynthesis in bananas (Figs. 1B, 2B, 3A). Terra *et al.* (33) suggested that sucrose synthase was involved in sucrose accumulation in bananas, yet sucrose synthase activity was relatively low and SPS was not assayed in their experiments.

During banana ripening, SPS activities were not parallel when assayed under conditions of saturating substrate and limiting substrate (plus Pi) concentrations (Fig. 2B). After 4 d, SPS activity remained high in the presence of saturating substrate concentrations but decreased in the presence of limiting substrates (plus Pi) (Fig. 2B). The results are suggestive of a change in the activation state of the enzyme in a manner analogous to spinach leaf SPS (32). However, it is

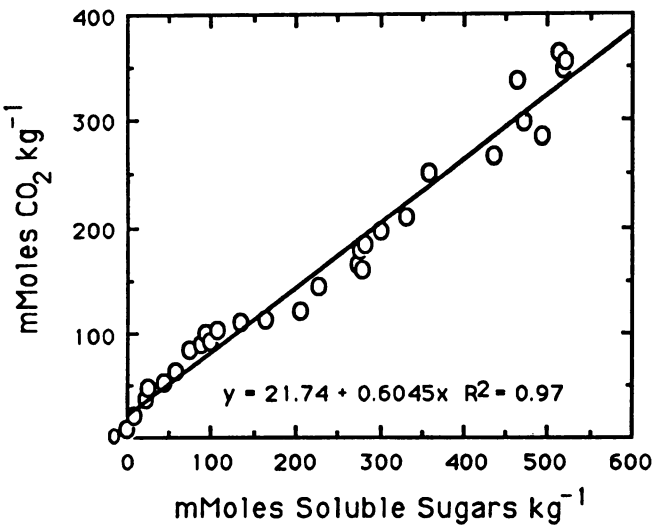

Figure 6. Correlation between cumulative CO_2 respired and total soluble sugars of banana fruit slices at short time intervals during ripening. Respiration and sugar concentration are each expressed as mmol sucrose equivalents $(kg)^{-1}$ fresh weight. Data were calculated from Figure 5, A and B.

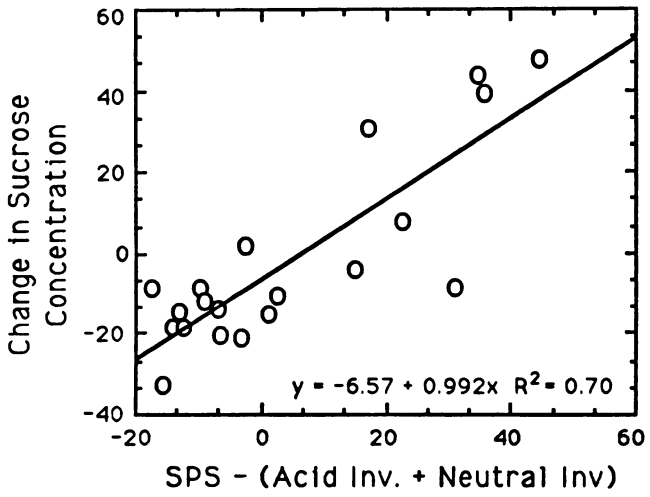

Figure 7. Relationship between the change in sucrose concentration and the mathematical difference between activities of SPS, measured with limiting substrates plus Pi, and the sucrose degrading enzymes, acid and neutral invertase in ripening banana fruit. Data are from individual replicates for each 48 h interval between d 2 and d 12.

not clear whether the affinity for hexose-phosphates and/or Pi was involved. It is tempting to speculate that if the kinetic properties of SPS change during ripening as a result of covalent modification that phosphorylation is involved. Phosphorylation has been reported to be a potential mechanism for regulation of activity of SPS from spinach leaf (16).

SPS has only recently been recognized as an important enzyme in some sink tissues that accumulate sucrose (10, 13). Of the sucrose metabolizing enzymes in muskmelon fruit mesocarp, activities of SPS and acid invertase changed during fruit development and were found to be primary determinants of sucrose accumulation (13). Sucrose accumulation in muskmelon fruit occurred only after SPS activity exceeded the sum of activities of all potential sucrose degrading enzymes. Enzyme activities and sucrose concentration changed in a different manner in ripening bananas than in developing muskmelon fruit; however, there was a similar relationship between fruit sucrose concentration and activities of sucrose metabolizing enzymes. In bananas, the change in sucrose concentration during consecutive sampling intervals tended to be positive when SPS activity (measured under conditions of limiting substrate concentrations) exceeded the sum of activities of the sucrose degrading enzymes, acid and neutral invertase (Fig. 7). The data in Figure 7 do not include the initial sampling date due to the uncertainty of the timing of the decline of sucrose synthase activity to undetectable levels between 0 and 2 d (Fig. 3A). The changes in enzyme activities and sucrose concentrations in both bananas and muskmelon fruit provide strong evidence that metabolic changes in fruit tissues during ripening are critical for sweetening to occur. In both instances a major change in SPS activity appeared necessary.

The observed increase in acid invertase activity (Fig. 3B) associated with a decline in sucrose concentration and increase in hexose sugar concentration (Fig. 1B) is consistent with previous findings (33). In addition, the increase in enzymatic capacity for sucrose hydrolysis during ripening is consistent with the postulate that hexose sugars arise from sucrose hydrolysis (5, 23, 33, 36).

Acid invertase is compartmentalized in the vacuole, the site of sucrose accumulation, and neutral invertase is located in the cytosol, the site of sucrose synthesis. The cellular locations of these enzymes, in addition to the carbohydrate data, suggests that concurrent sucrose synthesis and hydrolysis must occur. Huber (15) has suggested that there is substantial turnover of sucrose in mature leaf tissue of species that do not accumulate large quantities of sucrose during photosynthesis as a result of hydrolysis within the vacuole by acid invertase. Early in muskmelon fruit development, despite the presence of substantial SPS activity, there was no detectable sucrose accumulation (13). The lack of sucrose accumulation appeared to be the result of a greater capacity for degradation compared to synthesis of sucrose, and is suggestive of sucrose turnover. Furthermore, ripening muskmelon fruit on plants with reduced photosynthate supply appeared to synthesize sucrose at the expense of the fruit hexose pool (14). Thus, in various plant tissues including fruit, concurrent synthesis and degradation of sucrose may occur with a result of an increased energy requirement for net sucrose accumulation.

ATP Requirement of the Conversion of Starch to Sucrose

The conversion of starch to sucrose during banana ripening requires ATP which in harvested bananas is presumably supplied primarily through mitochondrial oxidative phosphorylation. In this study there was a strong positive linear correlation between the cumulative amount of CO_2 evolved and sugar accumulation throughout ripening (Fig. 6). Although such a correlation does not necessarily imply a causal relationship, it is well documented that sucrose does not accumulate in bananas in the absence of an increase in respiration (19, 22, 23, 24, 26). Together, these observations support a strong linkage between the climacteric and sugar accumulation.

Presuming that all soluble sugars accumulating in the banana arise from sucrose biosynthesis as supported herein, it is possible to calculate the ATP requirement for soluble sugar synthesis from starch. A minimum of one ATP is required for the synthesis of UDP-Glc (A substrate for SPS) by UDP-Glc pyrophosphorylase which is present in bananas throughout ripening (32). The UDP formed during sucrose biosynthesis must be rephosphorylated for continued sucrose formation. Additional ATP may be required depending upon the form of carbohydrate transported across the amyloplast envelope. Figure 8 illustrates two potential pathways for starch degradation in the amyloplast, transport across the amyloplast envelope, and subsequent synthesis of sucrose in the cytosol. The primary difference between the two pathways is the translocated form of carbohydrate out of the amyloplast. In cultured soybean cells (21) and cauliflower buds (18), triosephosphates cross the amyloplast in exchange for Pi. However, in amyloplasts from wheat endosperm, hexose-phosphates can also be translocated (20, 35). Borchert *et al.* (7) reported G-6-

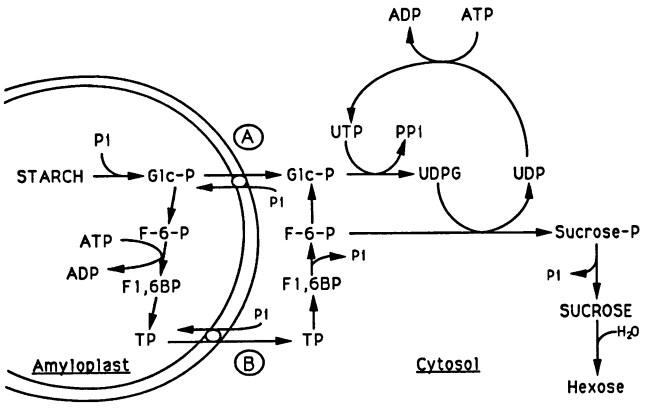

Figure 8. Potential pathways for starch degradation, transport across the amyloplast envelope and sucrose synthesis during banana ripening. In pathway (A) hexose-phosphates cross the amyloplast and in pathway (B) triose-phosphates (TP) cross the amyloplast.

P, triose-phosphates and 3-phosphoglycerate transport across pea root amyloplasts in a counter exchange for Pi. Unfortunately, there are no reports of similar investigations with banana amyloplasts. If glucose-phosphate, the product of phosphorylase activity, is transported out of the amyloplast in bananas, the demand for ATP in the conversion of starch to sucrose is one ATP per sucrose equivalent (Fig. 8). If on the other hand, triose-phosphates are translocated across the amyloplast envelope two additional ATPs are needed for the phosphorylation of Fru 6-P to fructose 1,6-bisphosphate (within the amyloplast) for a total of three ATPs for each molecule of sucrose synthesized. In the subsequent conversion of fructose 1,6-bisphosphate to Fru 6-P (in the cytosol) it is possible that PPi is generated through the reaction catalyzed by PPi-dependent phosphofructokinase (not illustrated). Any PPi generated in this manner, in addition to that generated by the reaction catalyzed by UDP-Glc pyrophosphorylase, could impact upon the overall energetics.

Respiration in bananas is thought to proceed primarily through the glycolytic pathway (5, 25). If the mitochondrial electron transport system were 100% efficient, the number of ATPs produced per CO_2 evolved would be six. A minimum of two ATPs per CO_2 evolved would result if all electrons flowed through the cyanide resistant pathway via alternate oxidase (9). In bananas, there appears to be little or no contribution to electron transport by the alternate oxidase during the climacteric (34).

According to the experimentally established relationship between CO_2 evolution and sugar accumulation, approximately 0.605 mol of CO_2 was respired per mol of soluble sugar (sucrose equivalent) accumulated in the tissue (Fig. 6). Based upon this relationship and the range of potential ATP produced (2–6 mol of ATP per mol of CO_2 respired) during respiration, the percentage of the potential ATP produced that was necessary to support the conversion of starch to sucrose was calculated. This relationship is depicted in Figure 9. If hexose-phosphates are exported from the amyloplast, the ATP requirement is 1 mol ATP per mol of sucrose and the requirement for ATP would be between 28 and 83% of the total produced (Fig. 9A). Transport of only triose-phosphates across the amyloplast would triple the ATP requirement to range from 83% to almost 250% of the theoretical production (Fig. 9B). Thus, with triose-phosphate transport from the amyloplast, the demand for ATP to support sucrose synthesis could only be met if mitochondrial oxidative phosphorylation were highly efficient. Furthermore, there would not be excess ATP to support turnover of sucrose which appears likely from the carbohydrate data and enzyme activities. These calculations present a strong argument that at least a portion of the carbohydrate exported from the amyloplast must be in the form of hexose-phosphates, particularly if sucrose turnover occurs. The data suggest that the conversion of starch to sucrose creates a far greater demand for ATP than that calculated by Solomos (30).

In summary, this study provides the first report of high SPS activity in bananas, as well as preliminary evidence for a change in kinetic properties of the enzyme during ripening. In catalyzing sucrose biosynthesis from starch, SPS creates a substantial demand for ATP during ripening. In addition, there appears to be a potential for sucrose turnover, a situation creating an even greater demand for ATP. The required ATP is supplied via mitochondrial oxidative phosphorylation. Sugar accumulation and respired CO_2 were highly correlated. Collectively, these data support the contention that sucrose

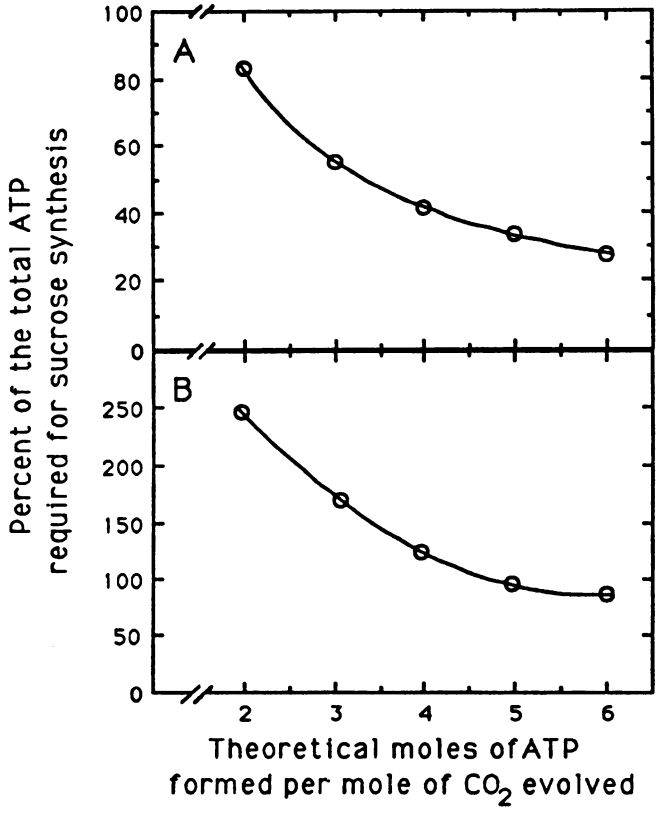

Figure 9. Percentage of ATP required for the starch to sucrose conversion in ripening banana fruit as a function of the theoretical mol of ATP formed (per mol of CO_2 evolved). Percentages are based upon (A) 1 mol of ATP and (B) 3 mol of ATP required per mol of sucrose synthesized. See text for discussion.

biosynthesis may contribute causally to the respiratory climacteric in banana fruit by creating rapid adenylate turnover. This is particularly true if there is a strong component of triose-phosphate export from the amyloplast as discussed above. There is a clear need for further information concerning the form(s) of carbohydrates which exit the amyloplast and the extent to which sucrose turnover occurs in bananas and other plant tissues.

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